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Small Angle X-ray Scattering of *E. coli trp* repressor: A Pilot Study

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Beamline: X27C

Introduction: Biophysical investigations of *E. coli trp* repressor (trpR) have lead to the hypothesis that the biologically relevant state of this L-tryptophan-activated DNA binding protein may be an aggregate rather than a single dimer (1). Our laboratory recently discovered that in aqueous alcohols trpR undergoes a dramatic conformational change and forms an ordered aggregate via domain-swapping (2). In pilot measurements at X27C in February 2001, SAXS concentration series measurements of purified trpR were made ± 2 mM L-tryptophan and ± 16% (v/v) isopropanol (in a base buffer of 100 mM HEPES, pH 7.5, 400 mM NaCl) to investigate a possible relationship between aggregation in physiological buffers and domain-swapping in alcohol.

Methods and Materials: Scattering data were collected on a Fuji image plate placed approximately 1700 mm from the sample, with fixed radiation wavelength = 1.366 Å. Scanned images were processed with POLAR (D. Fang, Stonybrook Technology and Applied Research) to obtain raw intensities for q in the range 0.08 to 2.6 nm-1. Model scattering curves were calculated using CRYSOL and distance distribution functions were calculated with GNOM (programs of D.I. Svergun, EMBL Hamburg).

Results: Sample scattering curves and Guinier fits are shown in Figure 1. Guinier-plot derived Rg values ranged from 8-20 nm, indicating that all samples were polydisperse, and contained aggregates (trpR dimer model Rg = 2.1 nm). Isopropanol was found to significantly increase Rg. Comparison of experimental and model distance distribution functions provided insight into aggregate morphologies (Figure 2). In the absence of alcohol, apo-trpR and holo-trpR distance distribution functions are consistent with the presence of compact tetramers and longer rod-like structures with similar cross-section. Addition of L-tryptophan loosens, but does not eliminate dimer-dimer association: periodic bumps in p(r) are suggestive of "beads-on-a-string." Upon addition of 16% isopropanol to either apo- or holo-trpR, distances that correspond to close interdimer contacts (3.5-4.5 nm) have strongly diminished contribution to scattering; instead, unassociated dimers appear mixed with larger, possibly spherical aggregates.

Conclusions: This pilot study has demonstrated that isopropanol disrupts a strong association between trpR dimers in solution. In addition, isopropanol significantly alters the morphology of trpR aggregates. These results suggest that the main mode of association between trpR dimers changes significantly upon addition of alcohol. Domain-swapping may therefore not be a relevant mode of trpR association under physiological conditions.

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References: 1. LeTilly, V. & Royer, C.A. Fluorescence anisotropy assays implicate protein-protein interactions in regulating *trp* repressor DNA binding. *Biochemistry* **32**, 7753-8. (1993). 2. C. Lawson, B. Benoff, H.M. Berman, J. Carey, T. Berger, manuscript in preparation

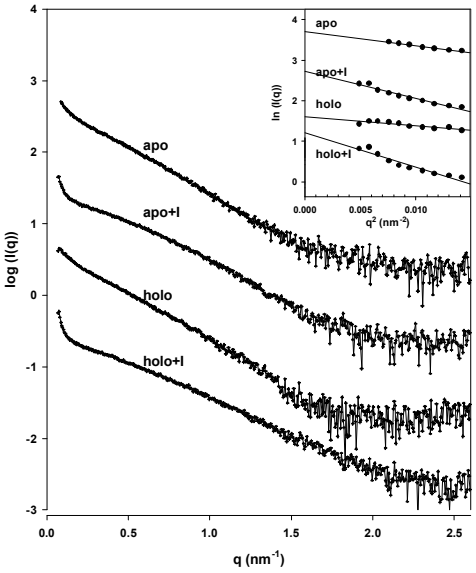

Figure 1. Sample trpR scattering curves. Apo = apo-repressor; holo=holorepressor (aporepressor + L-tryptophan); I=isopropanol

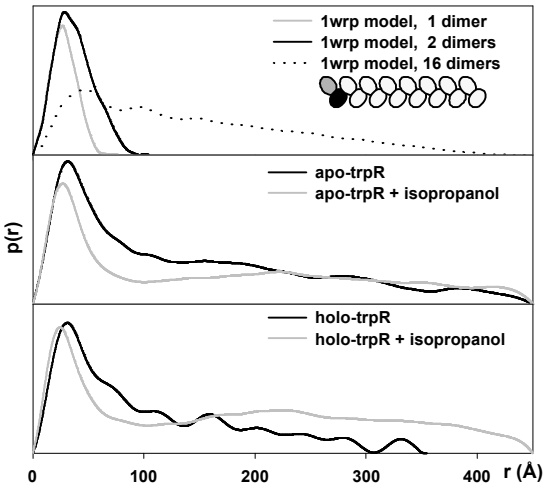

Figure 2. Distance Distribution functions. Top: Model functions for dimer, tetramer (=2 dimers), and a rod composed of 16 dimers. Middle and bottom: sample functions calculated from experimental curves.